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# Serum PARC/CCL-18 Concentrations and Health Outcomes in Chronic Obstructive Pulmonary Disease

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**Rationale:** There are no accepted blood-based biomarkers in chronic obstructive pulmonary disease (COPD). Pulmonary and activation-regulated chemokine (PARC/CCL-18) is a lung-predominant inflammatory protein that is found in serum.

**Objectives:** To determine whether PARC/CCL-18 levels are elevated and modifiable in COPD and to determine their relationship to clinical end points of hospitalization and mortality.

**Methods:** PARC/CCL-18 was measured in serum samples from individuals who participated in the ECLIPSE (Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints) and LHS (Lung Health Study) studies and a prednisolone intervention study. **Measurements and Main Results:** Serum PARC/CCL-18 levels were higher in subjects with COPD than in smokers or lifetime nonsmokers without COPD (105 vs. 81 vs. 80 ng/ml, respectively;  $P < 0.0001$ ). Elevated PARC/CCL-18 levels were associated with increased risk of cardiovascular hospitalization or mortality in the LHS cohort and with total mortality in the ECLIPSE cohort.

**Conclusions:** Serum PARC/CCL-18 levels are elevated in COPD and track clinical outcomes. PARC/CCL-18, a lung-predominant chemokine, could be a useful blood biomarker in COPD.

Clinical trial registered with [www.clinicaltrials.gov](http://www.clinicaltrials.gov) (NCT 00292552).

**Keywords:** biomarker; chronic obstructive pulmonary disease; PARC/CCL-18; chemokine

Chronic obstructive pulmonary disease (COPD) is a major health burden worldwide, affecting 10 to 15% of the adult population 40 years and older (1) and responsible for 3 million deaths annually (2). Although there has been rapid expansion in our understanding of COPD pathogenesis, therapeutic options

## AT A GLANCE COMMENTARY

### Scientific Knowledge on the Subject

Chronic obstructive pulmonary disease (COPD) is an independent risk factor for poor cardiovascular (CVD) health outcomes. However, there are no good blood-based biomarkers that can be used to predict these end points.

### What This Study Adds to the Field

The present study shows that PARC (pulmonary and activation-regulated chemokine)/CCL-18 (CC-chemokine ligand-18) associates with cardiovascular hospitalizations and mortality.

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remain limited. One major barrier to drug discovery has been the lack of reliable, robust, reproducible, and modifiable biomarkers that can act as intermediate surrogate end points for clinical studies (3). Although biomarkers can be obtained from any organ, the most attractive is blood because it is readily accessible and its measurements can be easily standardized. However, because most serum proteins are secreted by non-pulmonary organs such as the liver and the bone marrow, their use may be limited in COPD. This problem is largely avoided for proteins secreted predominantly by the lungs (i.e., pneumoproteins). PARC (pulmonary and activation-regulated chemokine)/CCL-18 (CC-chemokine ligand-18) is a 7-kD protein that is constitutively expressed by monocytes/macrophages and dendritic cells and is secreted predominantly in the lungs (4). Although the exact biological role of PARC/CCL-18 is not known, serum levels are elevated in acute coronary syndromes (5), with idiopathic pulmonary fibrosis (6), and in childhood acute lymphoblastic leukemia (7). Interestingly, in idiopathic pulmonary fibrosis, serum PARC/CCL-18 levels may reflect fibrotic activity and correlate with survival (8). Pertinent to COPD, in one small study of patients with mild to moderate disease, serum PARC/CCL-18 levels were significantly associated with reduced FEV<sub>1</sub> and with the BODE (body mass index, airflow obstruction, dyspnea, and exercise capacity) Index score (9), whereas in another, circulating PARC/CCL-18 levels were found to associate with acute exacerbations (10). Although these data are promising, several critical questions remain

unanswered regarding the possible use of PARC/CCL-18 as a biomarker in COPD, including its relationship with clinical outcomes such as hospitalization and mortality and its responsiveness to pharmacologic therapy. Using two large COPD cohorts, we determined the relationship of serum PARC/CCL-18 levels with lung function and mortality and determined whether PARC/CCL-18 levels can be down-regulated by prednisolone as a proof-of-concept that they can be modulated by an antiinflammatory drug. Some of the data have been published previously in abstract form (11).

## METHODS

See the online supplement for the details on methods.

### Subjects and Cohorts

For this study, we used data from three COPD cohorts: (1) the Lung Health Study (LHS, NCT00000568), (2) Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE), and (3) a prednisolone intervention study. The details of these cohorts have been previously published (12–14) and can be found in the online supplement. LHS enrolled smokers between the ages of 35 to 60 years who had mild to moderate airflow obstruction as defined by an FEV<sub>1</sub> of less than 90% but at least 55% of predicted, in the presence of an FEV<sub>1</sub>/FVC ratio less than 0.70. At the fifth annual visit, venipuncture was performed on 4,825 LHS participants, representing 89% of subjects known to be alive at Year 5. During the follow-up, the participants' vital status was captured semiannually. Vital status was successfully determined for 98.3% of the participants (15). On the basis of adjudication by an expert panel, mortality end points in LHS were classified as follows: malignancies; respiratory failure; coronary heart disease (CHD); and cardiovascular disease (CVD), which included CHD as well as other CVDs such as stroke (15).

### ECLIPSE

ECLIPSE (NCT00292552, GlaxoSmithKline study no. SCO104960) is a 3-year multicenter longitudinal observational study to identify novel end points in COPD. Individuals aged 40–75 years were recruited to the study if they had a smoking history of at least 10 pack-years; a post-bronchodilator FEV<sub>1</sub>/FVC ratio not exceeding 0.7; and GOLD (Global Initiative for Obstructive Lung Disease) stage II (FEV<sub>1</sub>, 50–80% predicted), III (30% ≤ FEV<sub>1</sub>, <50% predicted), or IV (FEV<sub>1</sub> < 30% predicted) COPD (16). Smoking (≥10 pack-years) and nonsmoking (<1 pack-year) control subjects were enrolled if they had normal lung function (postbronchodilator FEV<sub>1</sub> > 85% predicted and FEV<sub>1</sub>/FVC > 0.7).

### Prednisolone Intervention Study

For the prednisolone intervention study (NCT00379730, GlaxoSmithKline study no. RES106087), 89 current and former smokers were recruited, aged 40 to 80 years, with postsalbutamol FEV<sub>1</sub> between 30 and 80% of predicted and with chronic bronchitis. Individual subjects were randomized to receive either placebo or prednisolone (20 mg/d) for 4 weeks followed by prednisolone at 10 mg/day for 1 week, and prednisolone at 5 mg/day for 1 week. All subjects were followed up 2 weeks after the end of treatment.

### Measurements

LHS was used as the derivation cohort and ECLIPSE was used as the replication cohort. In LHS samples, we measured PARC/CCL-18 and 26 other serum proteins, using a highly sensitive chemiluminescence multiplexed sandwich ELISA analyzer (SearchLight proteome array system; Pierce Biotechnology Inc, Rockford, IL) (13) (see the online supplement for the list of serum proteins). In the ECLIPSE study serum samples, PARC/CCL-18 levels were measured with a human CCL18/PARC DuoSet ELISA kit (R&D Systems, Minneapolis, MN). The prednisolone study was used as an interventional cohort and the study samples were assayed for PARC/CCL-18, using the same platform as for the LHS samples.

### Statistical Analysis

To determine the relationship of serum PARC/CCL-18 concentrations with total and cause-specific mortality and hospitalization in LHS, we used a Cox regression analysis in which serum PARC/CCL-18 levels were divided into five equal-sized groups. Covariates that were considered in this model included age, sex, body mass index (BMI), smoking status through the first 5 years of follow-up, O'Connor slope for bronchial responsiveness (17), pack-years of smoking, blood pressure at Year 5, and FEV<sub>1</sub> (percent predicted) at Year 5. Only the covariates that were significant according to the LASSO (least absolute shrinkage and selection operator) method were included in the final model (see Table 3). The same covariates were used in the replication study using ECLIPSE.

## RESULTS

### Serum PARC/CCL-18 in LHS

The baseline characteristics of subjects at the time of blood collection for both LHS and ECLIPSE are summarized in Table 1. In the LHS, there were 329 deaths (6.8% of the LHS subjects in whom PARC/CCL-18 levels were determined). Of the LHS

TABLE 1. BASELINE CHARACTERISTICS OF LHS AND ECLIPSE COHORT

	Lung Health Study	ECLIPSE		
		Subjects with COPD	Smoker Control Subjects	Nonsmoker Control Subjects
Number of subjects	4,825	1,809	312	226
Age, yr	54 (7)	63 (7)	55 (9)	54 (9)
Men	3,036 (63%)	1,175 (65%)	172 (55%)	87 (39%)
White	4,644 (96%)	1,772 (98%)	305 (98%)	222 (98%)
Pack-years of smoking	40 (19)	48 (27)	32 (22)	0
BMI, kg/m <sup>2</sup>	26 (4)	27 (6)	27 (5)	28 (5)
FEV <sub>1</sub> , L	2.54 (0.67)	1.36 (0.52)	3.34 (0.76)	3.31 (0.80)
FEV <sub>1</sub> , % predicted	71.0 (12.4)	49 (16)	109 (12)	115 (14)
Intermittent smokers	1363 (28%)	N/A	N/A	0
Continuing smokers	855 (18%)	644 (36%)	192 (62%)	0
Chronic sputum*	1,477 (31%)	899 (50%)	54 (17%)	9 (4%)
Chronic cough*	1,697 (35%)	866 (48%)	60 (19%)	12 (5%)
PARC/CCL-18, ng/ml†	97 (34)	105 (26)	81 (21)	80 (18)

Definition of abbreviations: BMI = body mass index; COPD = chronic obstructive pulmonary disease; ECLIPSE = Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints; LHS = Lung Health Study; N/A = not available; PARC/CCL-18 = pulmonary and activation-regulated chemokine/CC-chemokine ligand-18.

Continuous data are expressed as means (SD) and dichotomous variables are expressed as number (percentage of column totals). Serum PARC/CCL-18 concentrations are expressed as medians (median absolute deviation).

\* At baseline.

† Median (median absolute deviation).

**TABLE 2. RELATIONSHIP BETWEEN SERUM PARC/CCL-18 CONCENTRATIONS AND TOTAL MORTALITY, CORONARY HEART DISEASE, AND CARDIOVASCULAR HOSPITALIZATION AND MORTALITY IN THE LUNG HEALTH STUDY**

End Point	PARC/CCL-18 as Continuous Variable ( <i>log scale</i> )	PARC/CCL-18 ( <i>quintiles</i> )				<i>P</i> Value for Trend
		Q2	Q3	Q4	Q5 ( <i>highest</i> )	
Total mortality	1.14 (0.93, 1.38)	1.16 (0.80, 1.70)	1.15 (0.80, 1.67)	1.24 (0.86, 1.79)	1.25 (0.87, 1.80)	0.27
Coronary heart disease deaths	1.26 (0.76, 2.08)	1.04 (0.32, 3.42)	2.44 (0.89, 6.72)	1.48 (0.50, 4.38)	1.52 (0.52, 4.43)	0.45
Cardiovascular deaths	1.20 (0.82, 1.77)	0.77 (0.34, 1.74)	1.10 (0.53, 2.30)	1.18 (0.57, 2.44)	1.05 (0.51, 2.17)	0.54
CHD hospitalization and deaths	1.33 (1.14, 1.56)	1.28 (0.90, 1.82)	1.63 (1.17, 2.26)	1.74 (1.25, 2.41)	1.83 (1.33, 2.53)	<0.01
CVD hospitalization and deaths	1.28 (1.13, 1.45)	1.15 (0.89, 1.49)	1.22 (0.95, 1.56)	1.53 (1.20, 1.94)	1.51 (1.19, 1.91)	<0.01

*Definition of abbreviations:* CHD = coronary heart disease; CVD = cardiovascular disease (which also includes CHD); PARC/CCL-18 = pulmonary and activation-regulated chemokine/CC-chemokine ligand-18; Q = quintile.

Each cell expresses an adjusted hazards ratio (95% confidence interval) using quintile 1 as the referent, adjusted for covariates selected by LASSO (least absolute shrinkage and selection operator) from age, sex, FEV<sub>1</sub> (percentage of predicted normal values), current smoking status, pack-years of smoking, body mass index, and other covariates (*see METHODS*).

subjects in whom PARC/CCL-18 levels were determined, 51 died (1.1%) of CHD, and 87 died of CVD (1.8%). In addition, 508 patients (10.5%) experienced a CHD hospitalization or CHD death and 820 (17.2%) experienced a CVD hospitalization or CVD mortality. In the LHS, PARC/CCL-18 was related to the risk of CVD hospitalization or mortality (adjusted hazard ratio [HR], 1.28; 95% confidence interval [CI], 1.13 to 1.45) and CHD hospitalization or mortality (adjusted HR, 1.33; 95% CI, 1.14 to 1.56). PARC/CCL-18 levels were not related to total mortality (Tables 2 and 3). PARC/CCL-18 was related to the patients' baseline FEV<sub>1</sub> percent predicted value (for every 10% predicted increase in FEV<sub>1</sub>, PARC/CCL-18 decreased by 3.0%;  $P < 0.0001$ ; *see* Figure E1 in the online supplement). PARC/CCL-18 levels were also related to patients' age (a 10-yr increase was associated with an 8.7% increase in PARC/CCL-18 levels), to BMI (a 1-kg/m<sup>2</sup> increase was associated with a 1.9% increase in PARC/CCL-18 levels), to sex (men had 10% greater PARC/CCL-18 levels than women), and to smoking status (all with  $P < 0.0001$ ). For the latter, continuing smokers had the lowest PARC/CCL-18 levels, whereas sustained quitters had the highest. Continuing smokers had PARC/CCL-18 levels that were on average 6% lower than those of sustained quitters ( $P = 0.005$ ).

#### Serum PARC/CCL-18 in ECLIPSE and Prednisolone Intervention Study

At study entry in ECLIPSE, serum PARC/CCL-18 levels were measured in 1,809 subjects with COPD, 312 smoking control subjects without airflow obstruction, and 226 nonsmoking control subjects. Median concentrations of serum PARC/CCL-18 were higher in the subjects with COPD compared with both control groups (105 ng/ml vs. 81 and 80 ng/ml in subjects with COPD, smoking control subjects, and nonsmoking control subjects, respectively;  $P < 0.001$ ) (Figure 1). However, there

were no significant differences in serum PARC/CCL-18 concentrations across the GOLD stages. As in the LHS, the median serum PARC/CCL-18 concentrations were higher in former than in current smokers both within subjects with COPD (111 vs. 96 ng/ml;  $P < 0.001$ ) and within smoker control subjects (89 vs. 75 ng/ml;  $P = 0.01$ ) (Figure E2). Similar observations were also noted in subjects with COPD when separated according to GOLD stages. Within a GOLD stage, the median PARC/CCL-18 concentrations were generally higher in former smokers than in current smokers (GOLD II: 111 vs. 97 ng/ml,  $P < 0.001$ ; GOLD III: 112 vs. 95 ng/ml,  $P < 0.001$ ; GOLD IV: 106 vs. 100 ng/ml,  $P = 0.12$ ). As in LHS, men had higher serum PARC/CCL-18 concentrations than women (COPD cohort, 108 vs. 100 ng/ml;  $P < 0.001$ ) and PARC/CCL-18 was associated with age (a 10-yr increase was associated with a 9% increase in PARC/CCL-18 levels) and BMI (a 1-kg/m<sup>2</sup> increase was associated with a 1.3% increase in PARC/CCL-18 levels). Of the 1,809 subjects with COPD with PARC/CCL-18 levels measured, 151 died (all causes) during the 3-year observation time (39, 76, and 36 subjects in GOLD stage II, III, and IV, respectively). The median serum PARC/CCL-18 concentrations in those who died was higher than in the 1,659 subjects with COPD alive at 3 years (122 vs. 104 ng/ml;  $P = 0.003$ ). Figure 2 depicts a Kaplan-Meier survival curve of subjects whose serum PARC/CCL-18 levels were above and less than or equal to 147 ng/ml, which represents the 95th percentile of nonsmoking control subjects (Figure 2). Adjusting for age, sex, BMI, and smoking status, the hazard ratio for PARC/CCL-18 levels above 147 ng/ml was 1.90 (95% CI: 1.34, 2.69;  $P < 0.001$ ). Importantly, in the ECLIPSE samples, the serum PARC/CCL-18 concentrations were stable over 1 year (data not shown). In the prednisolone interventional study, treatment with prednisolone resulted in a significant reduction in serum PARC/CCL-18 levels from 137

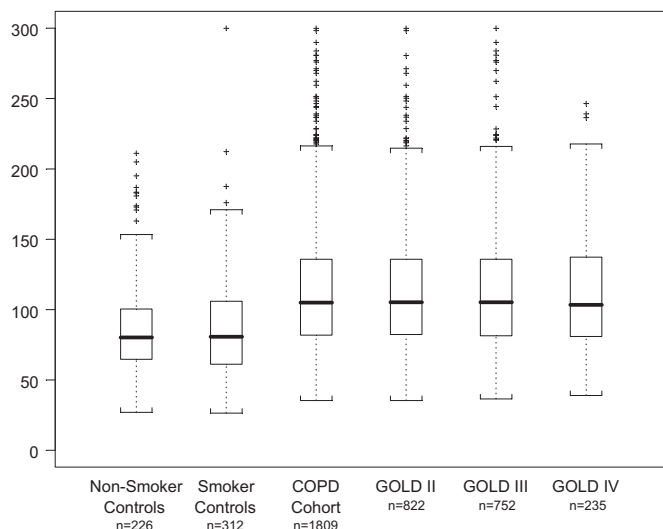
**TABLE 3. CLINICAL VARIABLES ASSOCIATED WITH SERUM PARC/CCL-18 CONCENTRATIONS IN LHS AND ECLIPSE PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE**

Change in Variable*	LHS		ECLIPSE COPD	
	Relative Ratio (95% CI)	<i>P</i> Value	Relative Ratio (95% CI)	<i>P</i> Value
10-yr increase in age	1.09 (1.06, 1.12)	<0.001	1.09 (1.07, 1.12)	<0.001
Males vs. females	1.10 (1.06, 1.14)	<0.001	1.05 (1.02, 1.09)	0.004
10-pack-year smoking increase	1.01 (1.00, 1.02)	0.068	1.00 (0.99, 1.01)	0.984
One unit change in BMI, kg/m <sup>2</sup>	1.02 (1.01, 1.02)	<0.001	1.01 (1.01, 1.02)	<0.001
FEV <sub>1</sub> increase of 10% predicted	0.97 (0.96, 0.98)	<0.001	1.00 (0.99, 1.01)	0.737
Continuing smokers vs. quitters†	0.95 (0.90, 0.99)	0.016	0.93 (0.90, 0.96)	<0.001

*Definition of abbreviations:* BMI = body mass index; COPD = chronic obstructive pulmonary disease; ECLIPSE = Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints; LHS = Lung Health Study; PARC/CCL-18 = pulmonary and activation-regulated chemokine/CC-chemokine ligand-18.

\* Change in individual variables holding other covariates fixed at their mean value.

† For ECLIPSE, this compares current smokers versus former smokers.



**Figure 1.** The relationship between serum PARC (pulmonary and activation-regulated chemokine)/CCL-18 (CC-chemokine ligand-18) concentrations and chronic obstructive pulmonary disease (COPD) in the ECLIPSE (Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints) study. ( $P < 0.001$  between COPD and control subjects; no difference between GOLD [Global Initiative for Obstructive Lung Disease] stages.)

to 98 ng/ml at 2 weeks ( $P < 0.001$  vs. placebo; see Figure E3). See the online supplement for additional results.

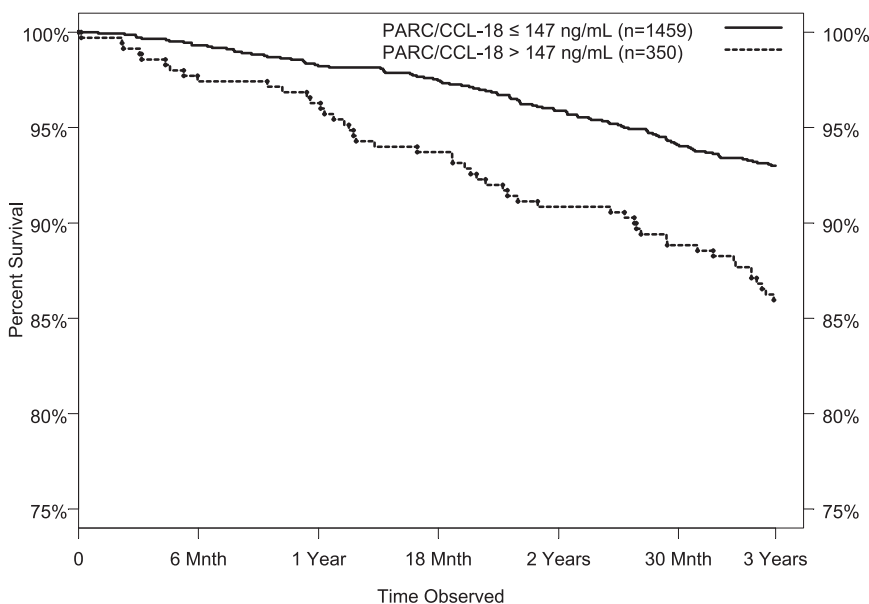
## DISCUSSION

Using two large COPD cohorts and an intervention study, PARC/CCL-18 appears to be a promising blood biomarker in COPD by fulfilling some of the important criteria of a biomarker, including its independent association with lung function, morbidity and mortality, and responsiveness to therapy (18). First, in the ECLIPSE study, we found that serum PARC/CCL-18 concentrations were independently related to COPD; whereas in the LHS, a large cohort of smokers with mild to moderate COPD (GOLD stages I and II), we found a significant

relationship with lung function, as measured by FEV<sub>1</sub> percent predicted. Second, we observed a strong, independent relationship of PARC/CCL-18 concentrations with future risk of cardiovascular hospitalization and mortality (in the LHS) and with total mortality (in ECLIPSE). Third, in the prednisolone interventional study, we found that serum PARC/CCL-18 levels could be modified by the short-term use of prednisolone, similar to what has been previously reported (19), which suggests that PARC/CCL-18 may be modifiable by systemic antiinflammatory therapy. Together, these data suggest that PARC/CCL-18 may be a useful candidate blood biomarker in COPD.

PARC/CCL-18 is an ~7-kD protein, which is expressed mainly by dendritic cells (20) and alternatively activated (M2) macrophages in the lungs (21) in response to helper T-cell type 2 cytokines. Minor expression has been noted in other inflammatory cells including peripheral mononuclear cells and neutrophils (22). Its precise function is not fully known. However, PARC/CCL-18 has been demonstrated to preferentially attract naive T cells to areas of injury, amplifying the initial inflammatory response (23). It can also stimulate lung fibroblasts to produce collagen by up-regulating the extracellular signal-regulated kinase-1/2 pathway (24). Serum concentrations of PARC/CCL-18 are elevated in patients with pulmonary fibrosis, which in turn correlate with increased fibrotic activity in the lungs and with reduced overall survival of these patients (8).

There is a paucity of data evaluating the role of PARC/CCL-18 in COPD. In a cross-sectional study, Pinto-Plata and colleagues showed that serum PARC/CCL-18 levels correlated significantly with reduced FEV<sub>1</sub> percent predicted values, increased the risk of exacerbation, and increased BODE Index, indicating poor prognosis (9). In another study, Hurst and colleagues demonstrated that during exacerbations, plasma PARC/CCL-18 levels increased by 10% compared with those during clinical stability (10), suggesting that this cytokine may be up-regulated during disease flare-ups and may be used as a biomarker of exacerbations. However, it should also be noted that although PARC/CCL-18 expression occurs mostly in the lungs, other organs including the prostate, bone marrow, blood vessels, and the liver also express this protein. Interestingly, serum PARC/CCL-18 levels rise during ischemic myocardial events and these levels predict future risk of cardiovascular morbidity and mortality (5). This latter observation may explain



**Figure 2.** Kaplan-Meier survival curves of ECLIPSE (Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints) patients with chronic obstructive pulmonary disease (COPD) stratified by serum PARC (pulmonary and activation-regulated chemokine)/CCL-18 (CC-chemokine ligand-18) concentrations (147 ng/ml represents 95th percentile of control subjects).

why in the LHS we observed a significant relationship between serum PARC/CCL-18 concentrations and the risk of cardiovascular hospitalization and mortality. Additional work will be needed to validate this early finding.

Although the LHS and the ECLIPSE study were conducted in different populations, across different time periods and with a different set of goals and objectives, there was remarkable consistency and complementarity of PARC/CCL-18 findings between the two studies. In both studies, serum PARC/CCL-18 concentrations were significantly related to increasing age, male sex, BMI, and smoking status. Interestingly, in both studies, ex-smokers had higher serum expression than did current smokers. Moreover, in both studies, total smoking exposure (as measured by pack-years of smoking) was not significantly associated with serum PARC/CCL-18 concentrations, indicating the importance of current smoking (or nonsmoking) status in modulating this protein. These clinical data are consistent with those of Kollert and colleagues, who showed that smoking attenuates PARC/CCL-18 expression by alveolar macrophages (25).

However, there were some notable discordances between the findings in the LHS and those in ECLIPSE. First, in the LHS but not in ECLIPSE, serum PARC/CCL-18 levels were associated with FEV<sub>1</sub> percent predicted. One possible reason is that the LHS had almost twice the sample size of ECLIPSE, which may have provided sufficient statistical power to detect this relationship. Another possibility is that the LHS studied predominantly patients with mild to moderate COPD whereas, in ECLIPSE, there was nearly equal representation of patients in GOLD class II and III and also a large representation of patients from GOLD class IV. It may be that the relationship of PARC/CCL-18 is (inversely) linear in earlier disease severity but nonlinear in later stages. The discordance in the FEV<sub>1</sub> data also raises the possibility that the relationship between FEV<sub>1</sub> and serum PARC/CCL-18 levels observed in the LHS could have been spurious. Additional work in other cohorts will be needed to determine which of these hypotheses, if any, are valid. Third, in LHS, total mortality was not associated with serum PARC/CCL-18 concentrations, whereas in ECLIPSE, it was significantly related. Fewer than 7% of the LHS participants died during an average follow-up of 8 years (resulting in an annual mortality rate of less than 1%). Thus, we may have lacked sufficient statistical power to assess mortality in the LHS. In contrast, by sampling patients with greater disease severity, patients in ECLIPSE had a higher mortality rate than those in the LHS, with nearly 10% mortality over 3 years of follow-up (resulting in an annual mortality rate of approximately 3%), resulting in more robust statistical power for this end point.

There were some limitations to the present study. Because only one additional lung function measurement was obtained after blood collection in the LHS, we could not determine the relationship of serum PARC/CCL-18 levels to the rate of descent in FEV<sub>1</sub>. Second, there were insufficient numbers of cardiovascular hospitalizations in ECLIPSE to determine the relationship of this protein with cardiovascular events and in the LHS, we did not have information about significant prognostic variables such as the BODE Index. As such, the value of serum PARC/CCL-18 above and beyond the BODE Index and other composite scores in predicting health outcomes in COPD is uncertain. In ECLIPSE, there was only a loose relationship between BODE Index scores and PARC/CCL-18 levels. Third, blood samples for the LHS have been stored at  $-80^{\circ}\text{C}$  for more than a decade. Although the blood samples have been well preserved and not subjected to repeat freeze-thaw cycles, the effect of long-term storage on most serum proteins is uncertain. Nevertheless, it was reassuring that the serum PARC/CCL-18 levels in the LHS subjects were similar to those in the ECLIPSE

subjects. Fourth, in the LHS, we measured multiple proteins (27 in total) and we cannot discount the possibility that the positive associations of serum PARC/CCL-18 to clinical end points in this cohort may have been spurious, owing to multiple comparisons. However, replication of some (but not all) of the findings of the LHS in ECLIPSE was reassuring. Last, it should be noted that serum PARC/CCL-18 measurements have not been standardized and as such absolute levels may not be comparable across studies. Thus, the data are best interpreted in relative (rather than absolute) terms.

In summary, these large COPD cohorts demonstrate PARC/CCL-18 as a promising serum biomarker in COPD. It is up-regulated in COPD and independently associated with important health outcomes such as total mortality and cardiovascular morbidity and mortality. Additional work needs to be done to validate these findings in other cohorts and, most importantly, to determine whether or not this protein is in the causal pathway of disease pathogenesis.

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